

# Glycosylated Serum Protein (GSP) Content Assay Kit

**Note:** Take two or three different samples for prediction before test.

**Operation Equipment:** Spectrophotometer/Microplate reader

**Catalog Number:** BC4945

**Size:** 100T/96S

## Components:

Reagent	Size	Storage
Reagent I	Solution 5 mL×1	4°C
Reagent II	Solution 2 mL×1	4°C
Reagent III	Solution 30 mL×1	4°C
Reagent IV	Solution 2 mL×1	4°C
Standard	Powder×1	4°C

## Solution preparation:

Standard solution: Add 0.8 mL Reagent II to prepare the 10 mmol/L DMF standard solution before use. Dilute the 10 mmol/L standard solution into the 4 mmol/L standard solution with Reagent II for test.

## Product Description:

Serum glucose reacts non-enzymatically with the N-terminal amino group of albumin and other serum protein molecules to form a polymer ketamine structure. Under alkaline conditions, it reacts with nitrotetrachloroazole blue to produce formazan, a purple-red compound. Comparing the color at 530 nm wavelength, measuring its OD value. And comparing with DMF standard, the content can be obtained.

## Reagents and Equipment Required but Not Provided:

Spectrophotometer/microplate reader, desk centrifuge, transferpettor, mico glass cuvette/96 well flat -bottom plate, mortar/homogeniser, ice and distilled water.

## Procedure

### I. Sample preparation:

Serum (plasma): collecting bacteria or cells into the centrifuge tube. According to the ratio of Serum (plasma)volume (mL): Reagent I volume (mL) = 10:1. It is suggested to take about 0.2 mL serum (plasma) and add 0.02 mL of Reagent I. Mix thoroughly. Stay in 37°C for 30 min.

### II. Determination procedure:

1. Preheat spectrophotometer/microplate reader for 30 minutes, adjust wavelength to 530 nm, set zero with distilled water.
2. Standard solution: According to the ratio of Standard solution volume (mL): Reagent I volume (mL) = 10:1. It is suggested to take about 0.2 mL Standard solution and add 0.02 mL of Reagent I. Mix thoroughly. Stay in 37°C for 30 min.

3. Add reagents with the following list:

Reagent (μL)	Test tube	Blank tube	Standard tube	Standard blank tube
Sample	10	-	--	-
Distilled water	-	10	-	-
Standard	-	-	10	-
Reagent II	-	-	-	10
Reagent III	200	200	200	200
Stay in 37°C for 15 min;				
Reagent IV	10	10	10	10
Mix thoroughly. Take 200 μL to micro glass cuvette/96 well flat -bottom plat. Measure the absorbance at 530 nm. Record as $A_T$ , $A_B$ , $A_S$ , $A_{SB}$ . $\Delta A_T = A_T - A_B$ , $\Delta A_S = A_S - A_{SB}$ .				

Note: Blank tube and Standard tube only need to test 1-2 times.

### III. Calculations:

$$\text{GSP (mmol/L)} = C \times \Delta A_T \div \Delta A_S \times F$$

C: Concentration of Standard solution, 4 mmol/L;

F: Dilution times.

#### Note:

- After color development, please add Reagent IV immediately. It is recommended not to make too many samples at once.
- If the measured absorbance value  $A > 1.5$  or  $\Delta A > 1$ , it is recommended to dilute the sample before measuring, and multiply the dilution factor in the calculation formula; if the measured absorbance value is low or close to the blank OD value, it is recommended to increase the sample volume before performing the measurement.

### Experimental example

- Take 0.2 mL mouse plasma and standard solution. Add 0.02 mL of Reagent I. Mix thoroughly. Stay in 37°C for 30 min. Follow the measurement procedure. Calculate  $\Delta A_T = A_T - A_C = 0.252 - 0.044 = 0.208$ .  $\Delta A_S = A_S - A_{SB} = 0.227 - 0.01 = 0.126$ . Calculate the content of glycated serum protein in mouse plasma according to the formula:  
 $\text{GSP (mmol/L)} = C \times \Delta A_T \div \Delta A_S = 4 \times 0.208 \div 0.126 = 6.603 \text{ mmol/L}$
- Take 0.2 mL horse serum and standard solution. Add 0.02 mL of Reagent I. Mix thoroughly. Stay in 37°C for 30 min. Follow the measurement procedure. Calculate  $\Delta A_T = A_T - A_C = 0.1 - 0.044 = 0.056$ .  $\Delta A_S = A_S - A_{SB} = 0.227 - 0.01 = 0.126$ . Calculate the content of glycated serum protein in mouse plasma according to the formula:  
 $\text{GSP (mmol/L)} = C \times \Delta A_T \div \Delta A_S = 4 \times 0.056 \div 0.126 = 1.778 \text{ mmol/L}$